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Antioxidant Activities of Phenolic Derivatives from *Dipsacus asper* Wall. (II)

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Abstract – The six phenolic compounds isolated from the root of *Dipsacus asper*, 3,4-di-*O*-caffeoylquinic acid (**1**), methyl 3,4-di-*O*-caffeoyl quinate (**2**), 3,5-di-*O*-caffeoylquinic acid (**3**), methyl 3,5-di-*O*-caffeoyl quinate (**4**), 4-5-di-*O*-caffeoylquinic acid (**5**), methyl 4,5-di-*O*-caffeoyl quinate (**6**) were continuously evaluated for their antioxidant activity using superoxide radical scavenging and AAPH-mediated (LDL) oxidation assay. The results demonstrated that compounds **1** - **6** had remarkable antioxidant activities with the IC_{50} values ranging from 12.0 to 2.8 μM in superoxide radical scavenging. They also inhibited AAPH-mediated low-density lipoprotein LDL oxidation by the generation of thiobarbituric acid reactive substances (TBARS) with IC_{50} ranging from 6.7 to 8.7 μM.
Keywords – *Dipsacus asper*, Dipsacaceae, phenolic, superroxide radical scavenging, low-density lipoprotein

Introduction

In recent years, the physiological functionality of medicinal plants and foods have received much attention due to the increasing interest in human health and have been studied in vitro and in vivo by many researchers. The antioxidative action, one of the important physiological functions of medicines, is supposed to protect living organisms from oxidative damages, resulting in the prevention of various diseases such as cancer and diabetes. Furthermore, the antioxidative activities of many plants have been evaluated, and their antioxidants properties have been identified (Herrman, 1976). The occurrence of various kinds antioxidative phonelic compounds, including flavonoids, diarylheptanoids, have been shown to be powerful antioxidants in vitro (Vinson *et al.,* 1995), and the close relations of phenolics to the antioxidative activity have been reported (Osawa *et al*., 1994; Veliogu *et al*., 1998).

Dipsacus asper Wall (Dipsacaceae) is a perennial herb that natively distribute in China. The roots of this plant have been used in traditional Chinese and Vietnamese medicine as an analgesic, and anti-inflammatory agent, for enhancement of liver, and for the treatment of fractures (Namba, 1993; Loi, 2001). Previously, it is reported that triterpene glycosides and iridoid glycosides are major components of this plant (Kouno *et al*., 1990; Jung *et al*., 1993; Tomita and Mouri, 1996). In addition, pharmacological studies demonstrated that the saponins from *D. asper* possessed anticomplementary (Oh *et al*., 1999), antinociceptive and neuroprotective activities (Suh *et al*., 1996; Zhang *et al*., 2003). As a part of continuing studies on chemical constituents and antioxidant activities of *D. asper*, we have found that the root this plant contents phenolic compounds as caffeyol quinic derivatives (Hung *et al.,* 2006). These compounds were found to be potent scavengers of the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) and they also inhibited Cu^{2+}-mediated low-density lipoprotein (LDL) oxidation by increasing the lag time of conjugated dienes formation and inhibited the generation of thiobarbituric acid reactive substances (TBARS) in the dose-dependent manners (Hung *et al*., 2006). In this present study, we wish to report in detail the isolation, structure elucidation and antioxidant activity of these compounds using the NBT reduction method in superoxide radical scavenging assay as well as LDL oxidation which initiated by the thermo-labile radical indicator (AAPH).

Experimental

Plant material – The dried root of *Dipsacus asper*

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Wall was obtained from the local Yuseong Herbal market. The voucher specimen (No. CNU 1284) has been botanically identified by Professor KiHwan Bae and deposited in the herbarium of the College of Pharmacy, Chungnam National University, Daejeon, Korea.

Chemical reagents – Xanthine, xanthin oxidase, nitro blue tetrazolium (NBT), 2,2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH), caffeic acid, quercetin, were purchased from Sigma Chemical Co., USA. Organic solvents were purchased from DaeJung Chemical & Metals Co. Ltd, Korea.

General procedure – Column chromatographic packing materials: Kieselgel 60; 70 - 23, 230 - 400 mesh (Merck, Germany), YMC-gel (OSD-A, 12 nm, S-150 μm), precoated TLC: Kieselgel 60 and F_{254}, RP-18 F_{254} (Merck, Germany). Preparative MPLC was carry out on Yamazen MPLC system with pump 540; detector Prep UV-10V; ULTRA PACK ODS-S-50A column (11 × 300 mm). Melting point was taken on Electrothermal apparatus. The IR spectra were determined on a Hitachi 270 - 30 type spectrometer with KBr discs. The optical rotations were determined on a JASCO DIP-370 digital polarimeter. FAB-MS was taken in MeOH and obtained using a JEOL JMS-DX 300 spectrometer. ^{1}H-NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker-AM600 FT-NMR unit and chemical shift are expressed as δ values using TMS as an internal standard.

Extraction and isolation – The air-dried root of *Dipsacus asper* (2 kg) was extracted with MeOH at room temperature for 6 days and the solution was dried *in vacuo*. The MeOH extract (240 g) was resuspended in H_2O and partitioned with hexane, EtOAc and BuOH, sequentially. The EtOAc-soluble fraction (25 g), that showed antioxidant activity with IC_{50} values of 12.9 and 8.8 μg/mL, respectively, in the DPPH and LDL oxidation assays, was subjected to silica gel column chromatography (5 × 30 cm) using a $CHCl_3$-MeOH gradient (from 100 : 1 to 0 : 1) to yield 10 fractions (Fr. 1 - 10). Fr. 7 (1.2 g), which was obtained from $CHCl_3$-MeOH (6 : 1), was chromatographed over a silica gel column (3 cm × 30 cm), eluted with a mixture of $CHCl_3$-MeOH-H_2O (4 : 1 : 0.1), to give three subfractions (Fr. 7.1 - 7.3). Fr. 7.2 was subjected to YMC column chromatography (2 × 50 cm) eluting with MeOH-H_2O (3 : 2) to give three fractions (Fr. 7.2.1 - 7.2.3). Repeat chromatograph fraction Fr. 7.2.1 on an RP-18 column (1.5 × 30 cm) eluted with MeOH-H_2O (1 : 1) to yield compounds **5** (11 mg) and **4** (8 mg). Fr. 7.2.2 was subjected to MPLC on ODS column (11 × 300 mm, Yamazen), eluted with MeOH-H_2O (1 : 1.5) (flow rate: 2 mL/min, UV 254 nm) to afford compound **3** (17 mg, t_R 14 min) and **6** (7 mg, t_R 16 min). Further purification of fraction Fr. 7.2.3 using MPLC with the similar above conditions resulted in the isolation of compounds **1** (13 mg, t_R 20 min) and **2** (9 mg, t_R 25 min).

Compound 1 – Colorless plates; mp. 155 - 158 °C; IR ν_{max} (KBr): 3400 (OH), 3300 - 2800 (COOH), 2958 (CH), 1719 (C = O), 1523, 1458 (C = C) cm^{-1}; UV λ_{max} (MeOH): 219, 248, 293, 329 nm; FAB-MS *m/z* 517 [M + H]$^+$; ^{1}H-NMR (400 MHz, MeOH-d_4) δ : 2.21 (1H, dd, H-2*eq*), 2.27 (1H, dd, *J* = 6.0 Hz, H-2*ax*), 5.07 (1H, dd, *J* = 6.0 Hz, H-3), 5.57 (1H, ddd, *J* = 5.8 Hz, H-4), 4.32 (1H, br d, *J* = 7.8 Hz, H-5), 2.05 (1H, br d, *J* = 7.2 Hz, H-6*eq*), 2.07 (1H, br d, *J* = 4.0 Hz, H-6*ax*), 6.95 (1H, dd, *J* = 14.2 Hz, H-2'), 6.97 (1H, d, *J* = 3.1 Hz, H-2''), 6.68 (1H, d, *J* = 3.1 Hz, H-5'), 6.70 (1H, d, *J* = 7.2 Hz, H-5''), 6.86 (1H, d, *J* = 7.2 Hz, H-6'), 6.86 (1H, d, *J* = 16.0 Hz, H-6''), 7.56 (1H, d, *J* = 16.4 Hz, H-7'), 7.52 (1H, d, H-7''), 6.19 (1H, d, H-8'), 6.21 (1H, d, H-8''). ^{13}C-NMR (100 MHz, MeOH-d_4) spectral data were in accordance with published data.

Compound 2 – Colorless plates; mp. 133 - 135 °C; IR ν_{max} (KBr): 3368 (OH), 2927 (CH), 1710 (C = O), 1543, 1458 (C = C), 1273 (C-O) cm-1; UV λ_{max} (MeOH) 216, 246, 296, 327 nm; FAB-MS *m/z* 531 [M + H]$^+$; ^{1}H-NMR (400 MHz, MeOH-d_4) δ : 2.22 (1H, dd, H-2*eq*), 2.26 (1H, dd, *J* = 6.0 Hz, H-2*ax*), 5.07 (1H, m, H-3), 5.43 (1H, m, H-4), 4.30 (1H, dd, *J* = 7.7 Hz, H-5), 2.02 (1H, dd, *J* = 7.5 Hz, H-6*eq*), 2.06 (1H, dd, *J* = 4.2 Hz, H-6*ax*), 3.67 (3H, s, H-8), 6.98 (1H, dd, *J* = 15.3 Hz, H-2'), 6.96 (1H, d, *J* = 3.3 Hz, H-2''), 6.71 (1H, d, *J* = 3.3 Hz, H-5'), 6.69 (1H, d, *J* = 7.1 Hz, H-5''), 6.69 (1H, d, *J* = 7.1 Hz, H-6'), 6.89 (1H, d, *J* = 16.0 Hz, H-6''), 7.57 (1H, d, *J* = 16.4 Hz, H-7'), 7.47 (1H, d, H-7''), 6.36 (1H, d, H-8'), 6.23 (1H, d, H-8''). ^{13}C-NMR (100 MHz, MeOH-d_4) spectral data were in accordance with published data.

Compound 3 – Colorless plates; mp. 150 - 153 °C; FAB-MS *m/z* 517 [M + H]$^+$; ^{1}H-NMR (400 MHz, MeOH-d_4) δ : 2.23 (1H, dd, H-2*eq*), 2.28 (1H, dd, *J* = 6.0 Hz, H-2*ax*), 5.37 (1H, dd, *J* = 6.2 Hz, H-3), 3.92 (1H, dd, *J* = 7.6 Hz, H-4), 5.33 (1H, dd, *J* = 3.5 Hz, H-5), 2.09 (1H, dd, *J* = 7.3 Hz, H-6*eq*), 2.12 (1H, dd, *J* = 3.8 Hz, H-6*ax*), 7.01 (1H, dd, *J* = 15.3 Hz, H-2'), 7.01 (1H, d, *J* = 2.4 Hz, H-2''), 6.74 (1H, d, *J* = 2.4 Hz, H-5'), 6.72 (1H, d, *J* = 7.6 Hz, H-5''), 6.91 (1H, d, *J* = 7.6 Hz, H-6'), 6.91 (1H, d, *J* = 16.0 Hz, H-6''), 7.58 (1H, d, *J* = 16.3 Hz, H-7'), 7.54 (1H, d, H-7''), 6.32 (1H, d, H-8'), 6.23 (1H, d, H-8''). ^{13}C-NMR (100 MHz, MeOH-d_4) spectral data were in accordance with published data.

Compound 4 – Yellow gum; mp. 145 - 146 °C; FABMS *m/z* 531 [M + H]$^+$; ^{1}H-NMR (400 MHz, MeOH-d_4) δ : 2.25 (1H, dd, H-2*eq*), 2.27 (1H, dd, *J* = 6.2 Hz, H-2*ax*),

5.35 (1H, dd, J = 6.2 Hz, H-3), 3.92 (1H, dd, J = 7.7 Hz, H-4), 5.33 (1H, dd, J = 3.5 Hz, H-5), 2.08 (1H, dd, J = 7.5 Hz, H-6*eq*), 2.11 (1H, dd, J = 3.8 Hz, H-6*ax*), 3.67 (3H, s, H-8), 7.01 (1H, d, J = 13.3 Hz, H-2'), 6.98 (1H, d, J = 2.6 Hz, H-2''), 6.74 (1H, dd, J = 2.7 Hz, H-5'), 6.73 (1H, d, J = 7.2 Hz, H-5''), 6.92 (1H, d, J = 7.5 Hz, H-6'), 6.90 (1H, d, J = 16.0 Hz, H-6''), 7.55 (1H, d, J = 16.4 Hz, H-7'), 7.47 (1H, d, H-7''), 6.31 (1H, d, H-8'), 6.27 (1H, d, H-8''). ^{13}C-NMR (100 MHz, MeOH-d_4) spectral data were in accordance with published data.

Compound 5 – Yellow powder; mp. 161 - 162 °C; FABMS *m/z* 517 $[M + H]^+$; ^{1}H-NMR (400 MHz, MeOH-d_4) δ: 2.28 (1H, dd, H-2 *eq*), 2.32 (1H, dd, J = 6.2 Hz, H-2*ax*), 4.28 (1H, dd, J = 7.4 Hz, H-3), 4.99 (1H, dd, J = 6.5 Hz, H-4), 5.55 (1H, dd, J = 5.5 Hz, H-5), 2.06 (1H, dd, J = 7.2 Hz, H-6 *eq*), 2.15 (1H, dd, J = 3.4 Hz, H-6 *ax*), 6.98 (1H, dd, J = 14.2 Hz, H-2'), 6.97 (1H, d, J = 2.1 Hz, H-2''), 6.72 (1H, d, J = 2.1 Hz, H-5'), 6.69 (1H, d, J = 7.3 Hz, H-5''), 6.87 (1H, d, J = 7.4 Hz, H-6'), 6.87 (1H, d, J = 16.0 Hz, H-6''), 7.52 (1H, d, J = 16.3 Hz, H-7'), 7.48 (1H, d, H-7''), 6.32 (1H, d, H-8'), 6.21 (1H, d, H-8''). ^{13}C-NMR (100 MHz, MeOH-d_4) spectral data were in accordance with published data.

Compound 6 – Yellow powder; mp. 140 - 143 °C; FAB-MS *m/z* 531 $[M + H]^+$; ^{1}H-NMR (400 MHz, MeOH-d_4) δ: 2.22 (1H, ddd, H-2*eq*), 2.29 (1H, ddd, H-2*ax*), 4.29 (1H, dd, J = 7.3 Hz, H-3), 5.49 (1H, dd, J = 6.5 Hz, H-4), 5.06 (1H, dd, J = 5.4 Hz, H-5), 2.02 (1H, ddd, H-6*eq*), 2.20 (1H, dd, J = 3.8 Hz, H-6*ax*), 3.67 (3H, s, H-8), 7.01 (1H, d, J = 14.2 Hz, H-2'), 6.92 (1H, d, J = 2.1 Hz, H-2''), 6.74 (1H, dd, J = 2.1 Hz, H-5'), 6.71 (1H, d, J = 7.4 Hz, H-5''), 6.88 (1H, d, J = 7.4 Hz, H-6'), 6.85 (1H, d, J = 16.0 Hz, H-6''), 7.57 (1H, d, J = 16.3 Hz, H-7'), 7.47 (1H, d, H-7''), 6.26 (1H, d, H-8'), 6.19 (1H, d, H-8''). ^{13}C-NMR (100 MHz, MeOH-d_4) spectral data were in accordance with published data.

Xanthine/Xanthine Oxidase assay – The assay was carried out basically according to the method of Beauchamp and Fridovich (Paya *et al*., 1992; Masaki *et al*., 1995) with some modifications. The 495 µL assay mixture consisted of 50 mM sodium carbonate buffer (pH 10.2), 0.1 mM xanthine and 25 µM nitro blue tetrazolium (NBT). The reaction was initiated by addition of 5 µL 20 nM xanthine oxidase in the presence or absence of each compound. The increase in absorbance at 560 nm was read after 5 min on a spectrophotometer (Shimadzu UV-1240, Japan). Superoxide radical scavenging activity was expressed by the degree of NBT reduction decrease of test group in comparison with that of the control group and calculated by the following equation:

$$\text{Scavenging activity (\%)} = [(Ac - As)/(Ac - Ab)] \times 100$$

Where Ac was the absorbance of the control, As was the absorbance of the sample and Ab was the absorbance of the blank (where xanthine oxidase was not added). The IC_{50} values were defined as the concentration that cause 50% decrease in NBT reduction.

LDL preparation – Blood was drawn from healthy normolipidemic volunteers and human LDL was prepared from plasma by sequential flotation ultracentrifugation as described previously (Kerry and Abbey, 1998). Briefly, plasma was centrifuged at 43,800 rpm for 20 h at 4 °C in Beckman T8-M ultracentrifuge and then chylomicron and very low-density lipoprotein floating to the top of tube was removed. Other supernatants were collected, and adjusted to d = 1.063 g/mL using NaBr and centrifuged at 43,800 rpm for 28 h at 4 °C. The top layer of LDL was purified by fast protein liquid chromatography on a Sephacyl S4000HR column (16 × 600 mm, Pharmacia Co.) using 10 mM Tris-HCl buffer (pH 7.4) containing 150 mM NaCl as the eluting solvent in the presence of 1 mM EDTA. The LDL was dialyzed against 50 µM phosphate-buffered saline (PBS) containing 1 µM EDTA for 24 h. EDTA was removed by a Sephadex G-25 column equilibrated with PBS. The purity of LDL evaluated by agarose gel electrophoresis was > 97%. The LDL protein was determined by the bicinchoninic acid method using bovine serum albumin as a standard (Smith *et al*., 1985).

Determination of TBARS – The oxidation of LDL to malondialdehyde (MDA) was measured using the thiobarbituric acid reactive substances (TBARS) assay (Jeong *et al*., 2004). Briefly, LDL (100 µg/mL) in PBS (pH 7.4, final volume of 1 ml) was pre-incubated with samples, and then 10 mM AAPH was added to initiate the oxidation. The reaction mixture was incubated at 37 °C for 3 h and the reaction was terminated by adding 250 µL of 20% trichloroacetic acid (TCA) and 250 µL of 1% thiobarbituric acid (TBA). After boiling at 95 °C for 5 min, the mixture was centrifuged at 10,000 rpm for 10 min. The absorbance of supernatant was measured at 532 nm. BHT, caffeic acid, quercetin, vitamin C, and vitamin E were used as reference antioxidants.

Results and Discussion

The EtOAc fraction of the MeOH extract of the root of *D. asper* was found to strongly exhibit antioxidant activity in superoxide radical scavenging assay with inhibition of 87.2% at the concentration 100 µg/mL. This suggested that compounds which, are presented in EtOAc

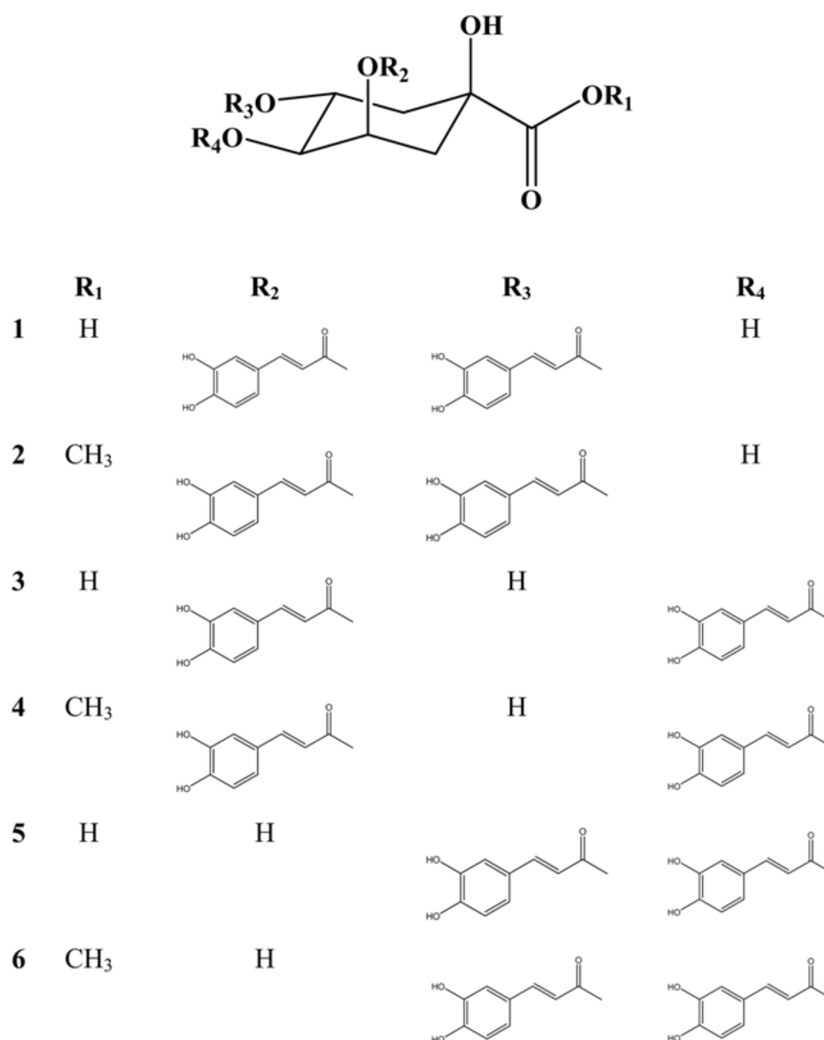

Fig. 1. Chemical structures of isolated compounds **1** - **6**.

fraction, possess prominent antioxidant activity. Repeated column chromatography of this fraction led to the isolation of six phenolic compounds (**1** - **6**). According to mass spectra fragmentation patterns and the products from hydrolysis experiments, compounds **1**, **3,** and **5** exhibited $[M+H]^+$ peaks at *m/z* 517, which was indicated of the molecular weight of 516 corresponding to dicaffeoyl quinic acid; compounds **2**, **4**, and **6** exhibited $[M+H]^+$ peaks at *m/z* 531 which was indicated of the molecular weight of 530 corresponding to methyl dicaffeoyl quinic acid (Chuda *et al*., 1998; Kwon *et al*., 2000; Soh *et al*., 2003). The ^{1}H- and ^{13}C-NMR of all compounds revealed the presences of two caffeoyl groups and a quinic acid moiety; two pairs of geminal protons, H-2 (*ax, eq*) and H-6 (*ax, eq*), were symmetrical positions and observed between δ 2.02 and 2.29 ppm; H-3, H-4, and H-5 protons in the quinic acid moiety were observed between δ 3.92 and 5.57 ppm. Of the double doublet signals, the downfield shift at the lowest frequency region was assigned to H-5 (δ 4.32 ppm with **1** and δ 4.30 ppm with **2** which was connected to hydroxyl group. The other two signals H-3 (δ 5.07 ppm) and H-4 (δ 5.57 ppm) of **1**, H-3 (δ 5.07 ppm) and H-4 (δ 5.43 ppm) of **2** indicated that the hydroxyl groups were substituted with the quinic groups. H-3 and H-4 occupied symmetrical positions in the planar structure. Moreover, because H-3 and H-4 had different stereochemical configurations (*ax* or *eq* given by the chirality), they were able to be distinguished by their coupling patterns. The ^{13}C-NMR data of **1** revealed the presences of a quinic acid moiety characterized with two methylenes at C-2 (δ 39.2 ppm) and C-6 (δ 38.3 ppm); three oxymethines at C-3 (δ 69.2 ppm), C-4 (δ 75.5 ppm) and C-5 (δ 68.9 ppm); one quaternary carbon (C-1, 75.9 ppm) and one carboxyl group (C-7, δ 176.5 ppm). Thus, **1**

was identified as 3,4-di-*O*-caffeoyl quinic acid. The ^{1}H-NMR and ^{13}C-NMR data of **2** were similar to those of **1** except that one methoxyl group occurred at C-8 (δ 53.1 ppm), H-8 (δ 3.67 ppm, s) and the chemical shift of C-7 (δ 175 ppm) was observed at a lower frequency. This substitution indicated that **2** was methyl 3,4-di-*O*-caffeoylquinate (Chuda *et al*., 1998). In the ^{1}H-NMR of **5** and **6**, the lowest downfield shift assigned to H-3 (δ 4.28 ppm and 4.29 ppm); the others were δ 4.99 ppm (H-4), δ 5.55 ppm (H-5) of **5** and δ 5.49 ppm (H-4), δ 5.06 ppm (H-5) of **6**. Moreover, the appearance of methoxyl group and the change-over chemical shift of C-7; **3**, **4**, **5**, **6** were established to be 3,5-di-*O*-caffeoyl quinic acid; methyl 3,5-di-*O*-caffeoylquinate; 4,5-di-*O*-caffeoyl quinic acid; methyl 4,5-di-*O*-caffeoylquinate, respectively. In addition, two caffeoyl groups of all compounds were observed as a pair of nonequivalent signals with coupling constants approximately 16.0 Hz, that confirmed for the *trans* olefinic structures (Chuda *et al* ., 1998; Yoshihiro *et al*., 1996; Kwon *et al*., 2003) (Fig. 1).

All six isolated compounds were tested for their superoxide radical scavenging capacity. As the results showed in Table 1, it is demonstrated that all the isolates (**1** - **6**) strongly inhibited against generation of superoxide anion with the IC_{50} values ranging from 12.0 μM to 12.8 μM. The similar activity of six quinic acid derivatives indicated that the methyl groups of **2**, **4**, and **6** did not play any role in scavenging capacity. The activity were much stronger than that of caffeic acid (IC_{50}, 45.6 μM), a positive control having the same structure with compounds **1** - **6** and vitamin C (IC_{50}, 45.6 μM). However, all the isolates were less potent than quercetin (IC_{50}, 5.2 μM), and apigenin (IC_{50}, 2.4 μM), which were also used as other positive controls. The oxidation of LDL initiated by Cu^{2+} was measured by the formation of malondialdehyde (MDA) using the TBARS assay. As shown in Table 1, compounds **1** - **6** tested remarkably reduced the formation of TBARS. They exhibited significant inhibitory activity against AAPH-mediated LDL oxidation with IC_{50} values of 8.1, 6.7, 8.2, 8.7, 7.5, and 6.9 μM, respectively. In this experiment, quercetin, vitamin C, and vitamin E used as reference antioxidants, they showed the inhibitory activity in Cu^{2+}-mediated LDL oxidation with IC_{50} values of 7.7, 25.5, and 5.8 μM, respectively. Vitamin C was less effective, meanwhile vitamin E inhibited stronger than that of isolated compounds, **1** - **6**. Previously, the antioxidant properties of some related compounds have been also reported (Azuma *et al*., 1999). Since quinic acid derivatives exhibited remarkable superoxide radical scavenging, it is conceivable that these natural products could be beneficial in preventing gout disease. This also might explain the traditional uses of *D. asper* as an agent in ethno-medicine.

Table 1. Antioxidant activities of compounds **1** - **6** against superoxide scavenging and AAPH-mediated LDL oxidation

Compounds	IC_{50} (μM) [a]	
	Superoxide scavenging	AAPH-mediated LDL [b]
1	12.3 ± 1.4	8.1 ± 0.4
2	12.0 ± 0.8	6.7 ± 0.6
3	11.4 ± 1.5	8.2 ± 0.5
4	12.8 ± 0.6	8.7 ± 0.5
5	12.5 ± 1.4	7.5 ± 0.2
6	11.7 ± 1.1	6.9 ± 0.3
Caffeic acid [c]	45.6 ± 5.8	ND
Catechin [c]	12.1 ± 1.4	ND
Apigenin [c]	2.4 ± 0.2	ND
Quercetin [c]	5.2 ± 0.5	7.7 ± 0.6
Vitamin C [c]	45.6 ± 5.0	25.5 ± 2.1
Vitamin E [c]	ND	5.8 ± 0.4

[a] IC_{50} values were determined by regression analysis and expressed as mean ± S.D. of three replicates.
[b] The inhibitory activity was measured using the TBARS assay.
[c] Positive controls.
ND: not determined.

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References

Azuma, K., Nakayama, M., Koshioka, M., Ippoushi, K., Yamaguchi, Y., Kohata, K., Yamaguchi, Y., Ito, H., and Higashio, H., Phenolic antioxidants from the leaves of *Corchorus olitorius* L. *J. Agric. Food Chem.* **47**, 3963-3966 (1999).

Chuda, Y., Suzuki, M., Nagata, T., and Tsushida, T., Contents and cooking loss of three quinic acid derivatives from Garland (*Chrysanthemum coronarium* L.). *J. Agric. Food Chem.* **46**, 1437-1439 (1998).

Herrmann, K., Flavonols and flavones in food plants: a review. *J. Food Tech.* **11**, 433-448 (1976).

Hung, T.M., Na, M., Thuong, P.T., Su, N.D., Sok, D., Song, K.S., Seong, H.Y., and Bae, K., Antioxidant activity of caffeoyl quinic acid derivatives from the roots of *Dipsacus asper* Wall. *J. Ethnopharmacol.* **108**, 188-192 (2006).

Jeong, T.S., Kim, J.R., Kim, K.S., Cho, K.H., Bae K.H., and Lee, W.S., Inhibitory effects of multi-substituted benzylidenethiazolidine-2,4-diones on LDL oxidation *Bioorg. Med. Chem.* **12**, 4017-4023 (2004).

Jung, K.Y., Do, J.C., and Son, K.H., Triterpene glycosides from the roots of *Dipsacus asper. J. Nat. Prod.* **56**, 1912-1916 (1993).

Kerry N. and Abbey, M., The isoflavone genistein inhibits copper and peroxyl radical mediated low-density lipoprotein oxidation in vitro. *Atherosclerosis* **140**, 341-347 (1998).

Kouno, I., Tsuboi, A., Nanri, M., and Kawano, N., Acylated triterpene glycoside from the roots of *Dipsacus asper. Phytochemistry* **29**, 338-339 (1990).

Kwon, H.C., Jung, C.M., Shin, C.G., Lee, J.K., Choi, S.U., Kim, S.Y., and Lee, K.R., A new caffeoyl quinic acid from *Aster scaber* its inhibitory activity against human immunodeficiency virus-1 (HIV-1) integrase. *Chem. Pharm. Bull*. **48**, 1976-1978 (2000).

Kwon, Y.S., Kim, K,O., Lee, J.H., Son, S.J., W, H.M., Chang, B.S., and Kim, C.M., Chemical constituents of *Dipsacus asper* (II). *Kor. J. Pharmacogn.* **34**, 128-131 (2003).

Loi, D.T., Glossary of Vietnamese Medicinal Plants. Hanoi S&T Publishing (2001).

Masaki, H., Sakaki, S., Atsumi, T., and Sakurai, H., Active oxygen scavenging activity of plant extracts. *Biol. Pharm. Bull.* **18**, 162-166 (1995).

Namba, T., The Encyclopedia of Wakan-Yaku (Traditional Sino-Japanese Medicines) with Color Pictures Vol. I. Hoikusha Publishing Co., Osaka, pp. 185-186 (1993).

Oh, S.R., Jung, K.Y., Son, K.H., Park, S.H., Lee, I.M., Ahn, K.S., and Lee, H.K., In Vitro anticomplementry activity of hederagenin saponins isolated from roots of *Dipsacus asper. Arch. Pharm. Res*. **22**, 317-319 (1999).

Osawa, T., Katsuzuki, H., Kumon, H., Kawakishi, S., Yamashita, K., Nakayama, T., and Hara, Y., Protective role of phenolic antioxidants in dietary plants against oxidative damage. *International Congress Series* (*Frontiers of Reactive Oxygen Species in Biology and Medicine*) **1058**, 333-336 (1994).

Paya, M., Halliwell, B., and Hoult, R.S., Interactions of a series of coumarins with reactive oxygen species. *Biochem. Pharm.* **44**, 205-214 (1992).

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., and Klent, D.J., Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**, 76-85 (1985).

Soh, Y., Kim, J.A., Sohn, N.W., Lee, K.R., and Kim, S.Y., Protective effects of quinic acid derivatives on tetrahydropepeveroline-induced cell death in C6 glioma cells. *Biol. Pharm. Bull*. **26**, 803-807 (2003).

Suh, H.W., Song, D.K., Son, K.H., Wie, M.B., Lee, K.H., Jung, K.Y., Do, J.C., and Kim, Y. H., Antinociceptive mechanisms of Dipsacus Saponin C administered intrecerebroventricularly in the mouse. *Gen. Pharmac*. **27**, 1167-1172 (1996).

Tomita, H. and Mouri, Y., An iridoid glucoside from *Dipsacus asperoides. Phytochemistry* **42**, 239-240 (1996).

Velioglu, Y.S., Mazza, G., Gao, L., and Omah, B.D., Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem*. **6**, 4113-4117 (1998).

Vinson, J.A., Dabbagh, Y.A., Serry, M.M., and Jang, J.H., Plant flavonoids, especially tea flavonols, is powerful antioxidants using an *in vitro* oxidation model for heart disease. *J. Agric. Food Chem.* **43**, 2800-2802 (1995).

Yoshihiro, C., Hirosi, O., Mayumi, O.K., Tadahiro, N., and Tojiro, T., Structural identification of two antioxidant quinic acid derivates from Garland (*Chrysanthemum coronarium* L.). *J. Agric. Food Chem.* **44**, 2037-2039 (1996).

Zhang, J.Z., Qian, Y.H., Yang, J., and Yang, G.D., The herbal medicine *Dipsacus asper* Wall. extract reduces the cognitive deficits and overexpression of β-amyloid protein induced by aluminum exposure. *Life Sci*. **73**, 2443-2454 (2003).

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